125143

THE UNITED STATES OF AMERICA

TO ALL TO WHOM THESE PRESENTS SHALL COME:

UNITED STATES DEPARTMENT OF COMMERCE

United States Patent and Trademark Office

November 24, 2004

THIS IS TO CERTIFY THAT ANNEXED HERETO IS A TRUE COPY FROM THE RECORDS OF THE UNITED STATES PATENT AND TRADEMARK OFFICE OF THOSE PAPERS OF THE BELOW IDENTIFIED PATENT APPLICATION THAT MET THE REQUIREMENTS TO BE GRANTED A FILING DATE.

APPLICATION NUMBER: *60/513,301*
FILING DATE: *October 22, 2003*
RELATED PCT APPLICATION NUMBER: *PCT/US04/35297*

Certified by

Jon W Dudas

Acting Under Secretary of Commerce
for Intellectual Property
and Acting Director of the U.S.
Patent and Trademark Office

Express Mail Label No. EL 988602081 US

Please type a plus sign (+) inside this box → [+]

6-03
Approved for use through 7/31/2003.
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE

Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it displays a valid OMB control number.

PROVISIONAL APPLICATION FOR PATENT COVER SHEET

This is a request for filing a PROVISIONAL APPLICATION FOR PATENT under 37 CFR 1.53(c).

22161 U.S. PTO
60/513301
102203

INVENTOR(S)		
Given Name (first and middle [if any])	Family Name or Surname	Residence (City and either State or Foreign Country)
Karl Steven	Booksh	Gilbert, AZ
Anna	Prakash	Mesa, AZ

Additional inventors are being named on the ___ separately numbered sheets attached hereto

TITLE OF THE INVENTION (500 characters max)

MULTIFUNCTIONAL MOLECULARLY IMPRINTED POLYMERIC FIBER-OPTIC SURFACE PLASMA RESONANCE CHEMICAL SENORS ON FLUORESCENT LANTHANIDE TEST BED

Direct all correspondence to: **CORRESPONDENCE ADDRESS**

☑ Customer Number 26707

OR *Type Customer Number here*

☐ Firm or Individual Name					
Address					
Address					
City		State		ZIP	
Country		Telephone		Fax	

ENCLOSED APPLICATION PARTS *(check all that apply)*

☑ Specification *Number of Pages* 9
☑ Drawing(s) *Number of Sheets* 6
☐ Application Data Sheet. See 37 CFR 1.76
☐ CD(s), Number
☑ Other (specify) Return Postcard

METHOD OF PAYMENT OF FILING FEES FOR THIS PROVISIONAL APPLICATION FOR PATENT

☑ Applicant claims small entity status. See 37 CFR 1.27.
☐ A check or money order is enclosed to cover the filing fees.
☑ The Director is hereby authorized to charge filing fees or credit any overpayment to Deposit Account Number: 17-0055
☐ Payment by credit card. Form PTO-2038 is attached.

FILING FEE AMOUNT ($) $80.00

The invention was made by an agency of the United States Government or under a contract with an agency of the United States Government.

☑ No.
☐ Yes, the name of the U.S. Government agency and the Government contract number are: _____

Respectfully submitted,

SIGNATURE [signature]

Date 10/22/03

TYPED or PRINTED NAME Christine M. Meis

REGISTRATION NO. *(if appropriate)* 52,024

Docket Number: 130588.00059 PRC

TELEPHONE 602-229-5247

USE ONLY FOR FILING A PROVISIONAL APPLICATION FOR PATENT

This collection of information is required by 37 CFR 1.51. The information is used by the public to file (and by the PTO to process) a provisional application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.14. This collection is estimated to take 8 hours to complete, including gathering, preparing, and submitting the complete provisional application to the PTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. SEND TO: Mail Stop Provisional Patent Application, Commissioner for Patents, Alexandria, VA 22313-1450.

1786883

EXPRESS MAIL EL 988602081 US

I hereby certify that this correspondence list d below is being deposited with the United States Postal Service on the date set forth below as Express Mail in an envelope addressed to: Mail Stop Patent Application, Commissioner for Patents, POB 1450, Alexandria, VA 22313-1450.

Date of Signature and Deposit: October 22, 2003 By: _____
Tessa Ricar

CERTIFICATE OF MAILING PURSUANT TO 37 C.F.R. 1.10

Applicant: Karl Steven Booksh et al.
Serial No.: to be determined
Filed: 10-22-2003
Title: MULTIFUNCTIONAL MOLECULARLY IMPRINTED POLYMERIC FIBER-OPTIC SURFACE PLASMA RESONANCE CHEMICAL SENSORS ON FLUORESCENT LANTHANIDE TEST BED
Art Unit: to be determined
Examiner: to be determined
Docket: 130588.00059 PRO

Mail Stop Patent Application
Commissioner for Patents
POB 1450
Alexandria, VA 22313-1450

Type of Filing:

1) Certificate of Mailing
2) Provisional Application for Patent Cover Sheet
3) Patent application, nine pages
4) Drawings, six sheets
5) Return receipt postcard

MULTIFUNCTIONAL MOLECULARLY IMPRINTED POLYMERIC FIBER-OPTIC SURFACE PLASMA RESONANCE CHEMICAL SENSORS ON FLUORESCENT LANTHANIDE TEST BED

BRIEF DESCRIPTION OF THE FIGURES

[0001] FIG. 1 illustrates the fiber-optic surface plasma resonance sensor with a molecularly imprinted lanthanide based test bed;

[0002] FIG. 2-1 shows the protocol for molecular imprinting of PMP on a SPR probe for bulk polymerization from solution;

[0003] FIG. 2-2 shows the protocol used for molecular imprinting of PMP on a SPR probe for surface initiated polymerization;

[0004] FIG. 3 displays the surface initiated polystyrene growth monitored by ATR-FTIR; the C=C and C-H stretch vibration peaks typical of polystyrene increase over the period of time shown;

[0005] FIG. 4 displays PMP binding on polystyrene surface studied by ATR-FTIR; and

[0006] FIG. 5 displays SPR responses to 100 ppb PMP in direct assay; the solid is the MIP-SPR probe; the dotted line is the control-SPR probe.

DETAILED DESCRIPTION OF A PREFERRED EMBODIMENT

[0007] A monolayer of polymerization initiator is covalently attached to the surface of the Surface Plasmon Receptor (SPR) – fiber optic surface. The imprint molecule (target molecule) is present in a polymerizable lanthanide metal monomer complex where it occupies a well-co-coordinated site within the complex. Cross-linking the vinyl groups present on the complex to the growing monomer or polymer chain on the surface of the SPR probe creates a very thin layer of molecularly imprinted polymer suitable for SPR signal sensing. The effective distance of surface plasmon penetration is only a few hundred nanometers, hence a monolayer of molecularly imprinted polymer is critical for the performance of the sensor.

[0008] After extraction of the template molecules, complimentary cavities remain in the polymer, which will be available to detect any new imprint molecule in solution. The inclusion and exclusion of the target molecule into/out of the polymer layers creates a change in the

refractive index of the sensing material and is transduced by the evanescent field created by the surface plasma resonance. This is used in real time sensing.

[0009] The performance of the SPR sensor off-line will be optimized by using the lumimescent lanthanide signal transducers embedded in the polymer layer. The presence of a fluorescent molecule, such as a lanthanide, invokes a specific spectral signature during the binding and removal of the imprint target molecule. This spectral signature will be used to optimize the performance of the SPR sensor in terms of selectivity and sensitivity. SPR signal and luminescent signal from the fiber will be isolated by using two different excitation/emission wavelength ranges.

[0010] The presence of a lanthanide signal transducer cross-linked into a growing polymer chain enables the optimization of the SPR sensor to selectively respond to the binding or removal of a template molecule of interest. This increases the sensitivity and selectivity of the Surface Plasmon Sensor tot the real-time detect of the target molecule.

Fiber-Optic-based Surface Plasmon Resonance (SPR) Sensors for the detection of toxic nerve agents

Anna M. C. Prakash, Yoon-Chang Kim, Soame Banerji, Jean-Francois Masson, Karl S. Booksh*
Dept. of Chemistry and Biochemistry, Arizona State University, Tempe, Arizona, 85287

ABSTRACT

Analytical instruments capable of detecting nerve agents in battlefield conditions where speed, accuracy and ease of operation are a must in today's military. Fast detection and decontamination of nerve agents in very low concentrations is the primary focus of our research. The method presented here focuses on optimizing polymer stabilized sensing elements on the surface of SPR fiber-optic probes. A number of polymers & polymer supported metal complexes capable of reversibly binding to the species of interest & which have robust operation in hostile environments are incorporated with the fiber optic sensing elements. An optical technique, such as Surface Plasmon Resonance (SPR), better suited to rapid data collection without sample pretreatment is employed. The approach using polymer-based optical fibers with off-the-shelf SPR system components has been tested for the detection of Pinacolyl methylphosphonate (PMP), a simulant for nerve agent Soman. Surface initiated polymeric sensors have higher sensitivity toward detecting PMP than bulk-polymerized sensors.

Keywords: Fiber-Optic Surface Plasma Resonance, surface initiated polymerization, bulk polymerization, nerve agent sensor, Styrene, Soman.

1. INTRODUCTION

Molecularly imprinted polymers (MIPs) have gained wide spread interest in recent years [1, 2, 3] in the design and development of smart sensors. MIPs are capable of changing their optical characteristics in a predictable way in the presence of an imprint molecule and are less prone to suffer from changes in pH, temperature, and trace of impurities that can easily contaminate the sensing surface. MIPs are tailor-made recognition elements that introduce specific recognition characteristics and could provide a promising alternative to bio-molecule based recognition elements. Molecular imprinting is a well-explored subject. Yet, in the case of surface plasma resonance based fiber optic sensors, it is often a challenge to get very thin (50nm) layer of, structured, functional, molecularly imprinted polymer layer on the surface of the gold-coated fiber. In this paper, we report the growth and the testing of molecularly imprinted polymers on the surface of a fiber-optic based Surface Plasma Resonance sensor, tailored for the detection of nerve agents in solution. Pinacolyl methylphosphonate (PMP), $CH_3POOHOCHCH_3C(CH_3)_3$, the hydrolysis product of nerve agent Soman, is used in this research instead of the nerve agent, Soman, $CH_3POFOCHCH_3C(CH_3)_3$.

Currently, the majority of papers on MIPs describe polymers synthesized by radical polymerization of functional and cross-linking monomers having vinyl or acrylic groups and using non-covalent interactions [4]. Most of these MIP based sensors are produced by dip coating methods. In designing our sensor, we have used two different approaches. One approach is molecular imprinting from bulk polymerization solution, where the imprint molecule (PMP) is premixed into a solution of monomers, initiators and cross-linkers and under suitable conditions imprinted on the surface of the SPR fiber directly from a polymerization solution. The other approach is to use surface initiation, where, the polymerization initiator is first covalently linked to the surface of the SPR fiber to initiate polymer growth from the surface of the fiber. The imprint molecule PMP is present in a polymerizable metal complex such as [Europium(vinyl benzoate)$_n$PMP], where it occupies a well-co-coordinated site within the complex. Cross-linking the vinyl groups present on the complex to the growing polymer chain on the surface of the SPR probe could create a very thin layer of MIP suitable for SPR signal sensing. After extraction of the template molecules, complimentary cavities remain in the polymer, which will be available to detect any new PMP

molecule in solution. The inclusion & exclusion of the target molecule (PMP) into/out of the polymer layers creates a change in the refractive index of the sensing material and is transduced by the evanescent field created by the surface plasma resonance. Our goal is to develop a SPR based sensor for nerve agents that can be used in real time. The performance of the sensor off-line however, will be optimized by using the luminescent lanthanide signal transducers embedded in the polymer layer or other spectroscopic methods as an optimizing test bed for the MIP-SPR sensor.

1.1 The need for a SPR based nerve agent sensor

Analytical methods currently used for the detection of nerve agents include ion mobility spectrometry [5], HPLC-GC/MS [6], SAW [7], luminescence spectroscopy [8], enzyme based chemistry [9] and others. However 'matrix effects' such as humidity, temperature, and composition of air sample can easily influence the IMS detector response. SAW based sensors, although fast, respond to all organophosphates and are sometimes irreversible. HPLC-GC/MS requires extensive pre-analysis procedures. The enzyme chemistry used in field analysis today can take up to 20-30 minutes to respond and are not reusable. Luminescence based fiber optic sensors for nerve agent detection can take about 15 minutes to respond & have heavy optical components. SPR based sensors for detecting nerve agents that we design in our lab are designed with priority to the needs of a US soldier in an enemy territory or an emergency rescue unit in a hostile chemical environment. Any detection system used under such conditions will have to be fast, accurate, mobile, fiber-optic based to access remote locations. Hence, a detection system, such as a fiber optic based Surface Plasma Resonance (SPR) sensor, is better suited for rapid data collection without sample pretreatment and is capable of providing a signal that will caution, terminate or proceed with a search mission by military personnel or emergency rescuers.

Engineering surfaces at a molecular level can lead to well-defined functionality, with better macroscopic properties than those obtained by conventional methods of surface modifications. The conventional casting methods of creating functionalized surfaces is based on dip coating of a preformed polymer [10] or by selective adsorption of a diblock copolymer [11, 12]. However, stearic and entropic forces hamper the growth of nano-scale layers from solution once the surface is significantly covered with an initial layer of a polymer layer. Also, the conventional methods, in general, lead to non-uniform thin films, which could affect the functionality of the surface and poor surface coverage due to the formation of so called islands and mushrooms on the surface. Other well-known methods such as polyelectrolyte deposition, plasma deposition, and polymerization with Langmuir-Blodgett (LB) film suffer from the same disadvantages. The direct initiation of a polymer chain from the surface has been explored as an alternative method for creating functionalized surfaces [13, 14], by chemically grafted thermal initiators to the surface of a silicon wafer or a gold-coated glass surface. With most of the polymer-based sensors produced by dip coating methods reproducibility due to leaching of the imprint molecules trapped in the inner core layers over time is a serious concern. Additionally, in SPR based detection methods, molecular imprinting by dip coating will create very thick polymer layer affecting the sensitivity of the SPR sensor. The effective distance of surface plasmon penetration is only few hundred nanometers. Therefore, creating monolayers of molecularly imprinted functional polymers close to the sensing surface will allow greater access of the target materials to the sensing area and improve reproducibility of the sensor.

1.2 Theory of Surface Plasma Resonance

The evanescent wave from the light in total internal reflection through the fiber optic can excite a standing charge on the gold surface. The localized fluctuations of electron density on the surface of the metal are known as surface plasmon. The surface plasmon (SP) wave is modulated from the dielectric constant of the thin gold film and the dielectric constant of the molecules adsorbed on the surface and within 100nm of the surface as shown in the Figure 1. The light at a fixed wavelength and fixed angle will enter in resonance with the surface plasmon and the photon will be absorbed. This will be seen by a minimum in the reflection spectra. The position of the minima is indicative of the refractive index of the material on the surface.

2. EXPERIMENTAL SECTION

2.1 Chemicals Used

Unless specified, all chemicals were used as purchased without prior purification and freshly prepared as required. Pinacolyl methylphosphonate (PMP), styrene, methacrylic acid (MAA), ethylene glycol dimethacrylate (EDMA), vinyl benzoate, 11-mercaptoundecanol, 4,4'-Azobis(4-cyano-valeric acid), 2,2'-Azobisisobutyronitrile (AIBN), epichlorhydrin, ethanolamine, N-hydroxysuccinimide (NHS), N-(3-dimethylaminopropyl)-N'-ethyl-carbodiimide hydrochloride (EDC) were all purchased from Aldrich, and divinyl benzene was purchased from Fluka.

2.2 ATR-FTIR

Brunker Optics IFS 66 v/S Vacuum FTIR, with a resolution of $4cm^{-1}$ and number of scans is 1024. The Harrick Scientific GATR™ grazing angle infrared ATR accessory used in this study uses 65° grazing angle ATR to study adsorbed species and monolayers on semiconductor and metallic substrates such as gold. The GATR™ is optimized for high sensitivity to these types of samples & gives repeatable measurements. Its specially designed pressure applicator is optimized for delivering good contact between the sample and the Ge ATR crystal. The ATR-IR provides at least an order of magnitude increase in sensitivity relative to grazing angle methods hence it was used in this study.

2.3 SPR Equipment

The light source is a white LED with a maximum emission at 640nm, and the spectrometer is a JobinYvon SPEX 270M housing with an 1800 grooves/mm grating blazed at 450-850nm (Jobin Yvon Inc). The detector is a CCD camera from Andor technologies model DU420-BR-DD. The region of interest on the CCD is vertically binned to across a 40 pixels stripe. The acquired signal by the Andor Basic software is converted to a text file and processed with Matlab 6.5. The fiber optic jumper was made with a 200-micron diameter fused silica fiber with a polyimide coating (Polymicro). The fiber optic probes are made with 400-micron diameter silica fibers. The Cr and Au layers are deposited with a Cressington 208HR sputter coater.

2.4 Preparation of Fiber-Optic Surface Plasma Resonance probes

The development of fiber-optic based SPR sensor in our research lab is well documented [15]. The fiber is a 400-micron silica core with a TECS cladding and a TEFZEL buffer (Thor Labs) with a numerical aperture of 0.39. The tip of the optical fiber is polished flat with lapping films (Thor Labs). A mirror is affixed onto the tip of the fiber optic probe by sputtering, first a layer of Cr (5 nm) followed by a layer of Au (50 nm). The fiber is then mounted in connector polished to ensure good optical coupling with the fiber optic jumper. Finally, approximately 1 cm of cladding near the tip of the silica fiber is removed by rubbing the cladding with a wiper soaked in acetone and then Cr and Au are sputter coated in the sensing area. The fibers are divided for use with either surface initiated polymerization or bulk polymerization as described below.

2.4.1. Preparing Fiber-Optic SPR probes for bulk polymerization from solution

The gold coated surface of the SPR fiber-optic probes from section 2.4.were immersed in an ethanol / water (4:1, v/v) solution containing ally mercaptane (3.0 mM) and 1-butanethiol (1.5 mM) for 24 hrs. This process covers the gold surface of the SPR probe with short carbon chain molecules containing polymerizable vinyl terminal groups as shown in Figure 2-1. The modified SPR probes were thoroughly rinsed with ethanol and dried with N_2.

2.4.2. Preparing Fiber-Optic SPR probes for surface initiated polymerization

In order to create a covalently linked self-assembled monolayer of a polymerization initiator, [4,4'-Azobis(4-cyano-valeric acid)], on the gold-coated surface of the SPR fiber as shown in Figure 2-2, the probes from section 2.4, were processed in the following manner. The fiber was immersed overnight

with 0.005M 11-mercaptoundecanol, washed by ethanol and dried in a stream of N_2. The 11-mercaptoundecanol on the gold was then reacted with epichlorhydrin in a mixture of diglyme and NaOH for 4 hours to give a reactive epoxide terminal. The epoxide was reacted with ethanolamine. The amine terminal was reacted with 4,4'-Azobis(4-cyano-valeric acid) in the presence of EDC/NHS mixture. All reactions were reproduced on gold-coated glass surface and followed by ATR-FTIR to optimize reaction conditions, to ensure completion of the reactions and to confirm the binding of the polymerization initiator to the surface of the SPR fiber.

2.5 Complex preparation

[Europium(vinyl benzoate)$_n$PMP] complex, was synthesized by mixing one mole of europium, one mole of PMP and *n* moles of vinyl benzoate as coordinating ligands and adjusting the pH suitable for complexation [13]. 3-7 moles of vinyl benzoate were used to as ligating molecules to accommodate the 9 coordination of the Eu^{3+}. A number of ligands currently in study, suitable for cross-linking to the SPR probe are reported in detail [16]. A blank complex without PMP was also synthesized in a similar manner.

2.6 Preparation of molecularly imprinted SPR fiber optic probes

Two different polymers were used in this study. One is methacrylic acid and ethylene glycol dimethacrylate based polymer. The other is styrene and divinylbenzene based polymer. They were polymerized on the surface of gold-coated SPR fibers using thermally induced free radical based polymerization. The following sections explain the fiber surface modification in the case of surface initiated polymerization and in the case of bulk polymerization from solution.

2.6.1 Preparation of molecularly imprinted SPR probes from bulk polymerization

For methacrylic acid (MAA) based polymerization of MIP from solution, a solution was prepared with 3 mol % of PMP as template molecule, 16 mol % of MAA as a functional monomer, 81 mol % of EDMA as a cross-linking monomer and 10 mg of AIBN as an initiator, dissolved in 18 ml of acetonitrile as a porogenic solvent. The control polymer solution was prepared in a similar fashion, but without introduction of the template molecule (PMP). The two solutions (with and without PMP) were sonicated under N_2 for 30 minutes. The modified fibers from section 2.4.1 were directly dipped into to these solutions and maintained at about 60 C for 1-2 hrs. The polymers thus created on the SPR probes were swelled in methanol to remove un-reacted monomer and the imprinted molecule. Additionally the imprint molecule on the SPR probe was extracted in a batch mode, using 0.25 % nitric acid in methanol / water (1:1, v/v) (3 × 10 min and room temperature). Figure 2-1, Probe1, describes the surface modification.

For Styrene based polymerization of MIP from solution, a solution was prepared by adding 3-5 mol % of complex synthesized in section 2.5 (with imprint molecule), 10 mg of 4,4'-Azobis(4-cyano-valeric acid) as an initiator, 90-95 mol % styrene as a functional monomer & 1-2 mol % divinyl benzene as a cross-linker. Jenkins et al. demonstrated that lower levels of cross-linking monomer allow better accessibility to the site in real time monitoring [7]. The control polymer solution was formulated in a similar fashion, but using 3-5 mol % complex synthesized without the imprint molecule (section 2.5). The two solutions were sonicated under N_2 for 30 minutes. The modified fibers from section 2.4.1 were directly dipped into to these solutions and maintained at about 60 C for 1-2 hrs. The extraction procedures were carried out as described above. Figure 2-1, Probe 2, describes the surface modification in detail.

2.6.2 Preparation of molecularly imprinted SPR probes from surface initiation

For surface initiated polymerization, the initiator, 4,4'-azobis(4-cyano-valeric acid), was covalently attached to the surface of the fiber as described in section 2.4.2. Methacrylic acid & styrene-based solutions were prepared as described above in section 2.6.1 but without the inclusion of the initiator in these solutions. This allows polymerization to initiate from the surface of the SPR fiber. The two solutions were sonicated under N_2 for 30 minutes. The SPR probes reported in section 2.4.2, with the initiator attached to the fiber were allowed to react in these solutions and maintained at about 60 C for a period of about 1-2 hrs. Under these conditions, the initiator attached to the surface of the fiber dissociates forming free radicals, thus initiating a well-controlled polymerization at the surface of the SPR fiber and

also cross-linking the complex to the surface of the sensor. Control probes were made without the template molecules. The extraction procedures were carried out as described above in section 2.6.1. Figure 2-2, Probe 3 describes surface modification of the SPR probe with methacrylate-based system and Figure 2-2, Probe 4 describes surface modification of the SPR probe with styrene-based system.

3. RESULTS AND DISCUSSION

3.1. ATR-FTIR study

The reactions detailed in section 2.6 were reproduced on Au-coated glass surfaces for ATR-IR study. Figure 3 shows the initial formation of a monolayer of styrene on the surface in less than 15 minutes. Two other spectra, collected after 4 hours and 6 hours show typical IR peaks growing in the C-H and C=C stretch vibrations as can be seen in the spectrum of reference polystyrene (top). The ATR-IR was studied when the sensing elements [Europium (vinyl benzoate)$_n$PMP] or the imprint molecule (PMP), was added to the polymer mixture and allowed to cross-link or adsorb on the surface of the growing polymer network on the surface.

In the case of the PMP immobilized directly on the polymer, the reaction can be easily followed with ATR-IR as shown in Figure 4. The IR peaks due to P=O, P-O-C stretch are unique and easy to identify during the inclusion and the exclusion of the PMP molecule from the surface of the polymer, when it is directly immobilized on the surface. However, in the case of molecular imprinting with [Europium(vinyl benzoate)$_n$PMP] complex, due to the large size of the lanthanide ion & the monomers compared to the PMP molecule, IR methods will not be sufficient for optimization. In this case, changes in the luminescent signal of the europium ion due to the changes in its electronic environment are a better choice as a signal transducer for off-line optimization of the sensor. The changes in the electronic environment of the lanthanide with and without the PMP molecule can be very specific as shown by Jenkins et al. [13]. A number of lanthanide complexes are currently under study for this purpose for use with SPR probes and are discussed in our next publication in detail [16].

3.2. SPR study

In order to investigate the use of MIP-based SPR probes to determine PMP, the binding phenomena of molecularly imprinted materials on the modified surfaces were studied by using fiber-optic SPR spectroscopy. As described in section 2-6, two different approaches, one from bulk polymerization and the other from surface initiated polymerizations were used to prepare the MIP. The change in the SPR coupling wavelength caused by binding of the styrene-based polymer material from bulk polymerization was studied. The change in SPR coupling wavelength increases with increase in the polymerization time and indicated that the styrene-based polymer material was polymerized on the SPR probe. A similar, time-dependent, but large positive change in SPR coupling wavelength was observed during styrene-based polymerization with surface initiated polymerization. On the other hand, when the MAA-based polymerization was monitored with fiber-optic SPR spectroscopy, the SPR spectra were not observed due to the cut off in the range of the refractive index covered by the current SPR sensor. The refractive index of the polymer materials obtained by a.KRUSS OPTRONIC refractometer are as follows: Styrene-based polymer material, RI = 1.3348; MAA-based polymer material, RI = 1.4445. The current fiber-optic SPR sensor covers a refractive index range of 1.3298 – 1.4006.

Figure 5. shows the kinetics of adsorption of PMP on the four different SPR probes upon exposure to PMP solutions (100 ppb in methanol / water (1:1, v/v)) and subsequent solvent (methanol / water (1:1, v/v)) in a batch mode. Each graph has two signals: one is from imprinted polymer-coated SPR probe; the other one is from unimprinted polymer-coated SPR probe as a control. Additionally each graph has three regions: first and third regions indicate the SPR responses of the solvent; second region indicates the SPR coupling wavelength changes in 100 ppb PMP sample. The arrows indicate exchange point of samples. Unlike conventional flow system based SPR sensors, in the fiber optic based SPR systems, rapid negative / positive changes in SPR coupling wavelength is observed when exchanging MIP and control SPR probes to PMP / subsequent solvent solutions. This is due to the sensitivity of our SPR sensors to very little

differences in the concentration between the solvent of PMP sample and the assay running solvent. The rapid response is followed by slower increase / decrease as the PMP bound to the MIP polymers on the SPR probes.

The difference in the SPR signals during PMP binding on the probes made by bulk polymerization and surface initiated polymerization was investigated with probes 1 and 2 vs. probes 3 and 4. The density of MIP recognition sites present on the probe would affect the sensitivity of a SPR sensor. From the data shown, it can be seen that the wavelength shifts on Probes 1 & 2 are ~12 % of those obtained by Probes 3 & 4. Therefore, we can conclude that the density of recognition sites on the MIP grown from surface initiation is much more than that of bulk polymerization.

Concluding remarks

A simple and rapid method for the detection of PMP by SPR was developed. This method shows that molecularly imprinted polymers grown by surface initiation on SPR sensors can increase the sensitivity of the sensor by several folds. A detailed work increasing the selectivity of the SPR sensor by using the signal transduced from the lanthanide test bed on the MIP is currently in preparation. If this SPR fiber-optic sensor is optimized for both selectivity and sensitivity, the rapid detection of nerve agents by SPR methods may prove to be very useful in search mission by military personnel or emergency rescuers.

Reference

1. Takeuchi, T.; Fukuma, D.; Matsui, J., *Anal. Chem.*, **71**, 285-290, 1999.
2. Jenkins, A.L.; Yin, R.; Jensen, J.L., *Analyst*, **126**, 798-802, 2001.
3. Sergeyeva, T.A.; Piletsky, S.A.; Brovko, A.A.; Slinchenko, E.A.; Sergeeva, L.M.; El'skaya, A.V., *Anal. Chim. Acta.*, **392**, 105, 1999.
4. Haupt K., *Analyst*, **126**, 747-756, 2001.
5. Brletich, N. R.; Waters, M.J.; Tracy, M.F., *Worldwide Chemical Detection Equipment Handbook*; Chemical and Biological Defense Information Analysis Center: Aberdeen, MD, 1995.
6. (a) D'agostino, P. A.; Provost, L.R.; Brooks, P.W., *J. Chromatog.*, **541**, 121-130, 1991. (b) Black, R.M.; Clarke, R.J.; Reid, M. J., *J. Chromatog.*, **662**, 301-321, 1994. (c) Santesson, J. FOA *Briefing on Chemical Weapons*, Edgewood Arsenal, 1974.
7. (a) Nieuwenhuizen, M.S.; Harteveld, J.L.N., *Talanta*, **41**, 461-472, 1994. (b) Kepley, L.J.; Crooks, R.M.; Ricco, A., *J. Anal. Chem.*, **64**, 3191-3193, 1992.
8. Jenkins, A.L.; Uy., O.M.; Murray, G.M., *Anal. Chem.*, **71**, 373-378, 1999.
9. Trettnak, W.; Reininger; Zinterl, E.; Wolfbeis, O.S., *Sens. Actuators B*, **11**, 87-93, 1993.
10. (a) Tsubokawa, N.; Hosoya, M.; Yanadori, K.; Sone, Y., *J. Macromol. Sci. Chem.*, **A27**, 445, 1990. (b) Zajac, R.; Chakrabarti, A., *Phys. Rev. E*, **52**, 6536, 1995.
11. Hadziioannou, G.; Patel, S.; Granick, S.; Tirrell, M., *J. Am. Chem. Soc.*, **108**, 2869-2876, 1986.
12. Spatz, J.P.; MÖller, M.; Noeske, M.; Behm, R.J.; Pietralla, M., *Macromolecules*, **30**, 3874-3880, 1997.
13. (a) de Boer, B.; Simon, H. K.; Werts, M. P. L.; van der Vegte, E. W.; Hadziioannou, G., *Macromolecules*, **33**, 349-356, 2000. (b) Tsubokawa, N.; Hayashi, S., *J. Macromol. Sci. Chem.*, **A32**, 525, 1995. (c) Pucker, O.; RÜhe, J., *Macromolecules*, **31**, 602-613, 1998.
14. (a) Wittmer, J.P.; cates, m. E.; Johner, A.; Turner, M.S., *Europhys. Lett.*, **33**, 397, 1996. (b) Szleifer, O.; Carignano, A., *Adv. Chem. Phys.*, **94**, 165, 1996.
15. Obando, L. A.; Booksh, K.S., *Anal. Chem.*, **71**, 5116-5122, 1999.
16. Prakash, A.; Kim, Y.C.; Banerji, S.; Booksh, K.S., *In Preparation*, 2003.

What is claimed is:

1. A surface plasmon sensor comprising:

a polymer chain, and

a lanthanide signal transducer, cross-linked into the polymer chain.

2. The surface plasmon sensor of claim 1, wherein the sensor is capable of detecting the binding or removal of a molecule.

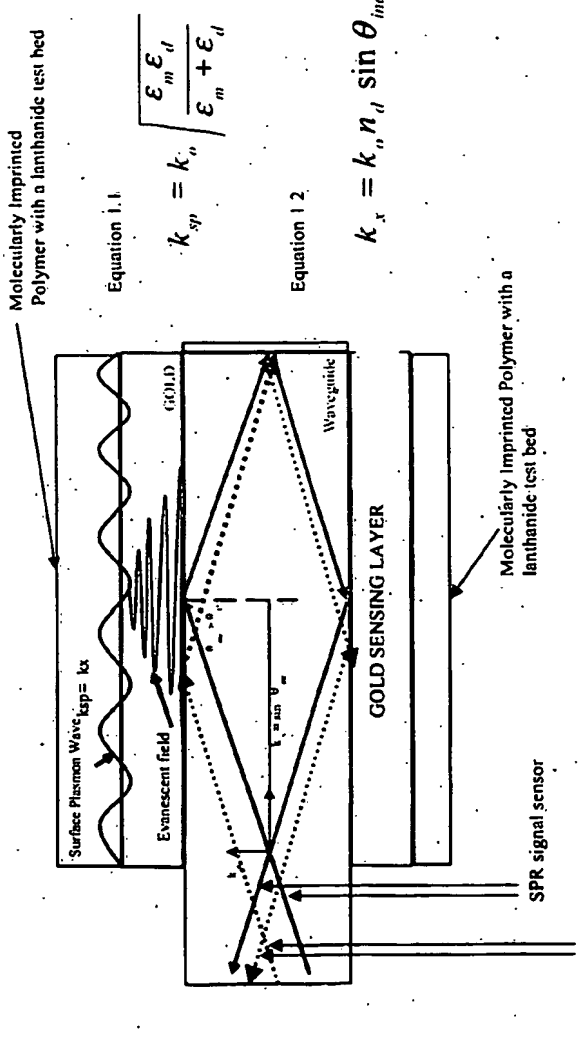

Fig. 1. Fiber-optic Surface Plasma resonance sensor with a molecularly imprinted Lanthanide based test bed

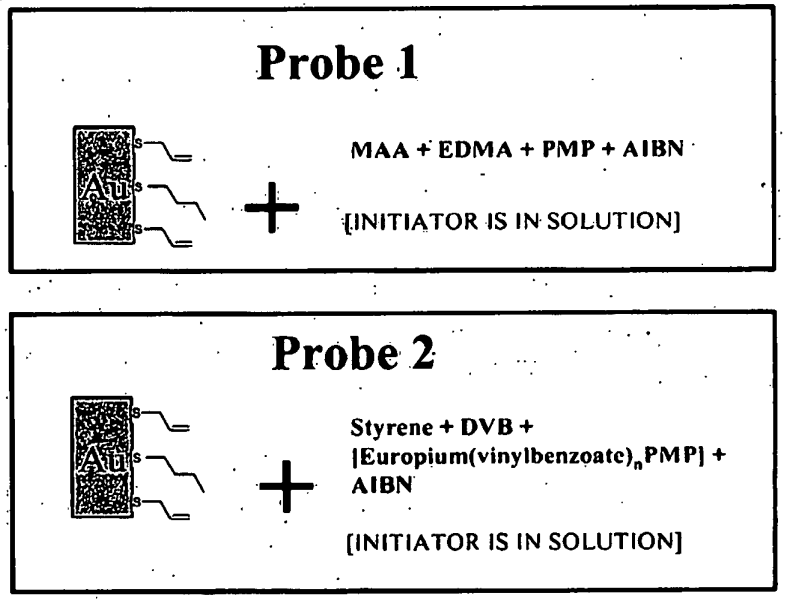

Fig. 2-1. Protocol used for Molecular imprinting of PMP on a SPR probe for bulk polymerization from solution

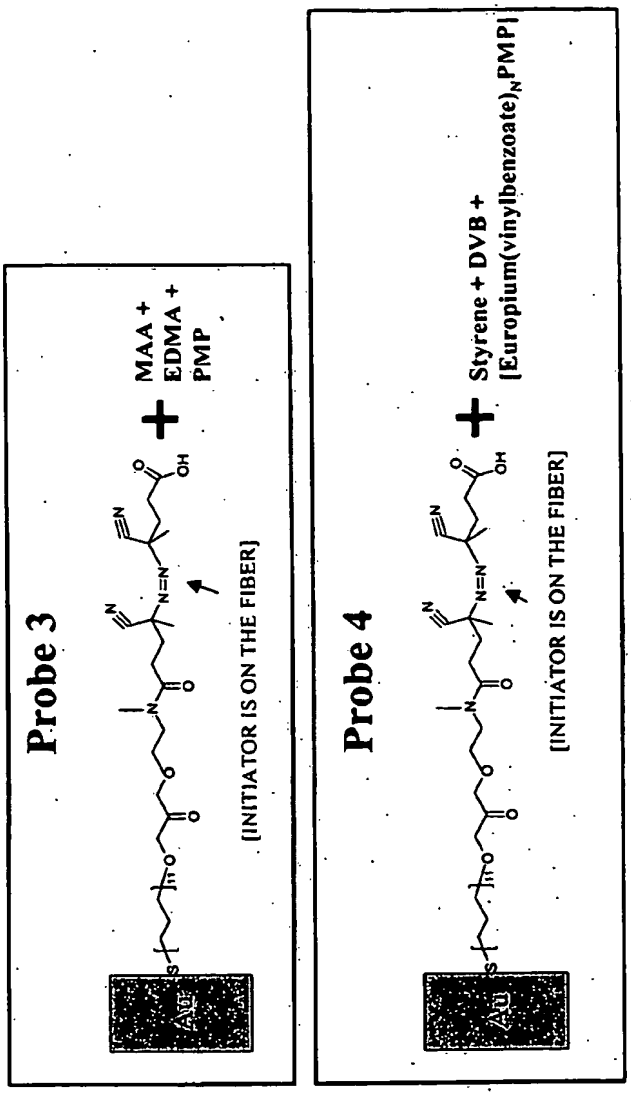

Fig. 2-2 Protocol used for Molecular imprinting of PMP on a SPR probe for surface initiated polymerization

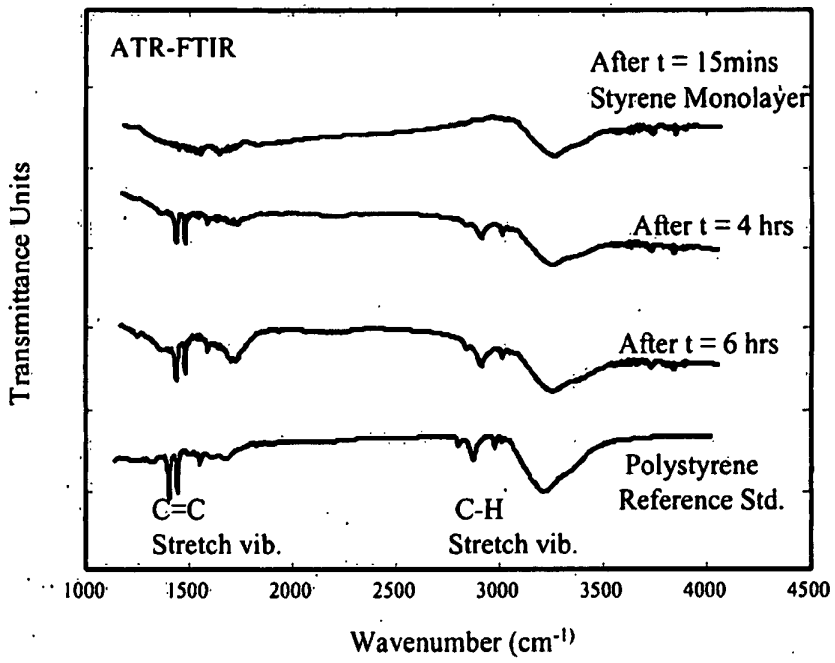

Fig. 3. Surface initiated polystyrene growth monitored by ATR-FTIR. The C=C & C-H stretch vibration peaks typical of Polystyrene grow over a period of time.

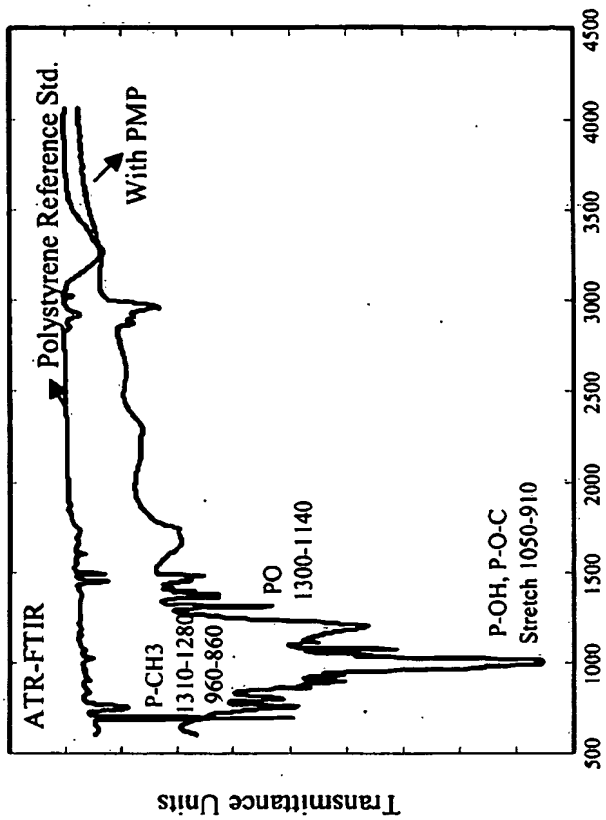

Fig. 4. PMP binding on polystyrene surface studied by ATR-FTIR

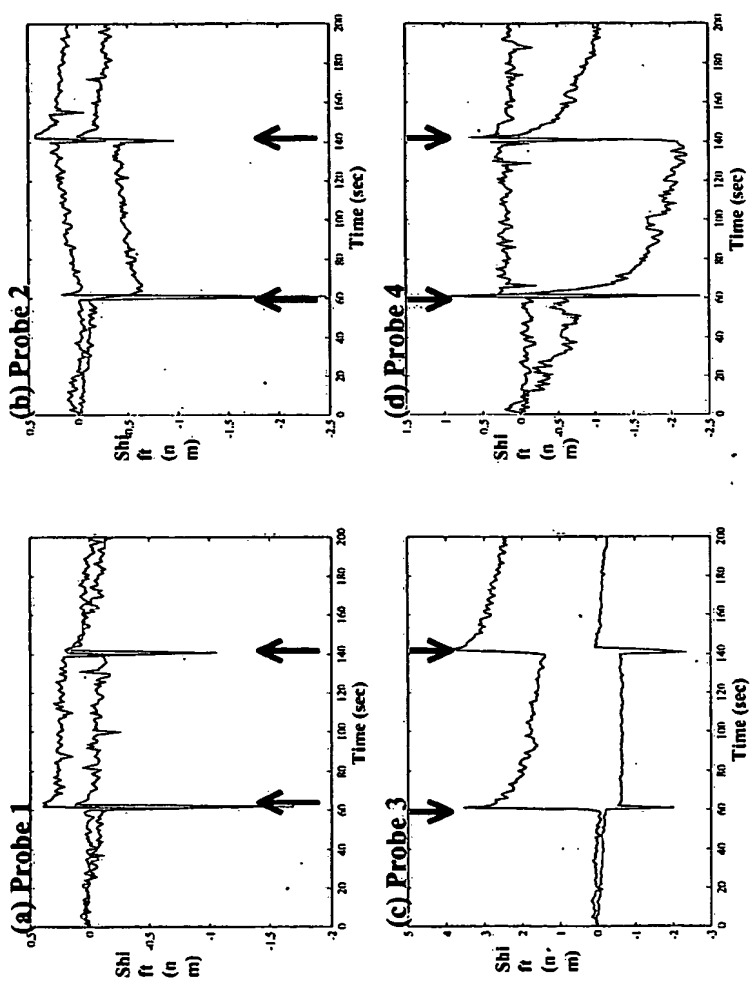

Fig5. SPR responses to 100 ppb PMP in direct assay: solid line, MIP-SPR probe; dotted line, control-SPR probe.

Document made available under the Patent Cooperation Treaty (PCT)

International application number: PCT/US04/035297

International filing date: 22 October 2004 (22.10.2004)

Document type: Certified copy of priority document

Document details: Country/Office: US
Number: 60/513,301
Filing date: 22 October 2003 (22.10.2003)

Date of receipt at the International Bureau: 06 December 2004 (06.12.2004)

Remark: Priority document submitted or transmitted to the International Bureau in compliance with Rule 17.1(a) or (b)

World Intellectual Property Organization (WIPO) - Geneva, Switzerland
Organisation Mondiale de la Propriété Intellectuelle (OMPI) - Genève, Suisse